Journal of Applied Botany and Food Quality 87, 206 - 209 (2014), DOI:10.5073/JABFQ.2014.087.029

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Influence of the extraction solvent on antioxidant capacity and total phenolic in currant fruits

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(Received March 31, 2014)

Summary

In black and red currant fruits the phenolic content and antioxidant capacity were determined. Two solvent systems (methanol and ethanol) at different concentrations and two methods of extraction were used. The study was conducted using fruits of black currant and red currant for determinations. The total phenolics content of each extract was measured according to the Folin-Ciocalteu method. The anti-oxidant capacity of the fruit extracts was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. It was found that the efficiency of the solvents used for the extraction of polyphenols varied substantially. The total phenolic content was 0.88 to 4.6 gallic acid equivalents in milligrams per gram of fresh weight (mg GAE /g FW). The content of phenolics was highly correlated with the anti-oxidant capacity (r = 0.97 - 0.98) and extracts obtained using ethanol solvents were more effective radical scavenging activities than the ones obtained using methanol solvents. Fruits of red and black currant represent an abundant source of phenolics, and prove to have good anti-oxidant capacity.

Abbreviations: DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE: gallic acid equivalents; TPC: total phenolics contents; TEAC: Trolox equivalent antioxidant capacity.

Introduction

Black currants (*Ribes nigrum*) and red currants (*Ribes rubrum*) are species of the genus *Ribes* broadly cultivated domestically and commercially as a temperate climate fruit crop. The currant is highly appreciated for the food and therapeutic value of its fruits (NOUR et al., 2011). Fruits of currant represent a source of natural anti-oxidant compounds. They contain high levels of phenolic compounds (eg. flavanols and anthocyanins), which substantially contribute to anti-oxidant activity (REMBERG et al., 2007; SLIMESTAD and SOLEIM, 2002). The HPLC analysis conducted by KAPASAKALIDIS et al. (2006) showed that delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside were the major anthocyanins and they constituted the main phenolic class (≈ 90%) in black currant residues that were tested. Fruits extracts from various black currant and red currant cultivars effectively act as free radical inhibitors (TABART et al., 2011; WANG and LIN, 2000). Research on anti-oxidant phenolic compounds in black currant was mainly focused on the effect of cultivars, seasonal timing and cultivation practices (TABART et al., 2006; NOUR et al., 2011). Some studies were carried out on total phenolics of currants. BORGES et al. (2010) and NOUR et al. (2011) have determined the phenolic, anthocyanin and ascorbic acid content of currant fruits. According to LUGASI et al. (2011), total polyphenol contents of white, red and black currants cultivars were 333, 192 and 533 mg/100 g, respectively. Black currants have high concentrations of flavonols and phenolic acids while in red currants the concentration of phenolic acids was higher than the flavonols concentration (JAKOBEK et al., 2007). GOPALAN et al. (2012) stated that black and red currants represent a good source of bioactive compounds with considerable dietetic and therapeutic impact on human health. In the present paper, total phenolics and anti-oxidant capacities in fruits of various black and red currant cultivars, which are commonly consumed in diet, were analyzed. Anti-oxidant capacities of different black and red currant cultivars and extracts were compared with the aim of preparing extracts with high anti-oxidant capacity.

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Materials and methods

Material

The study was conducted using fruits of black currant (cv. 'Black Down', 'Bogatar', 'Tenah', 'Record', 'Tinker', 'Deea', 'Abanos', 'Ronix') and red currant (cv. 'Houghton Castle', 'Abundent', 'Rosu timpuriu') for determinations. The study material was collected in Craiova, located in Southwest of Romania (44°20'N, 23°49'E), at the commercial maturity stage. The climate is temperate continental and plain specific, with Mediterranean influences. The average temperature range from 10 °C to 11.5 °C and precipitation level is 580 - 600 mm. Currant fruits were harvested from 10 bushes of each cultivar. Approximately 500 g of fruits were collected from each genotype. Fruits were stored on ice in the field and frozen at -10 °C, taking care to avoid unripe, damaged, or overripe fruits.

Total phenolics content

The fruits from each cultivar were homogenized using a laboratory blender. Extracts were prepared using two methods and two solvents. The sample (1 g) was extracted with 5 ml 80 % methanol (variant 1), 40 % ethanol (variant 2) or 80 % ethanol (variant 3) in ultrasonic bath for 55 minutes, centrifuged for 10 minutes at 5000 rpm, and the supernatant was filtered through polyamide filter, and finally transferred into vials for analysis and stored at 4 °C. In the variant 4, the sample (1 g) was extracted with 5 ml 40 % ethanol by maceration at room temperature for four weeks. The total phenolics content of each extract was measured according to the Folin-Ciocalteu method, described by SINGLETON and ROSSI (1965) with slight changes. Each 1.0 ml of fruits extract, or 1.0 ml double-distilled water (blank), 1 ml of each standard solution was placed in a 25 ml flask and 5 ml of Folin-Ciocalteu reagent was added (diluted 1:10 with ultrapure water). After 2 min, 4 ml of 7.5 % (w/v) sodium carbonate solution was added and flasks were kept at room temperature (24 °-26 °C) for 2 h. The absorbance was measured at 765 nm using an Evolution 600, UV-visible spectrophotometer (Thermo Scientific, USA) with computer control with VISION Pro-software (Thermo Scientific, USA). A standard curve was prepared by using 50, 100, 150, 200, or 250 mg/l gallic acid in 60:40 (v/v) methanol and water. Gallic acid was used as reference standard and total phenolic content were expressed as mg gallic acid per gram fresh weight (mg GAE/g FW).

Anti-oxidant activity

Methanol (Merck, Germany), DPPH (Merck, Germany) and Trolox (Merck, Germany) were used to determine anti-oxidant activity. The

scavenging activity of ethanolic and methanolic extracts of black and red currants against DPPH radicals was established in accordance with the method described by HATANO et al. (1988), with some modifications (COSMULESCU and TRANDAFIR, 2012). Each fruit extract (50 ml) was mixed with 3 ml of a 0.004 % (v/v) DPPH methanolic solution. Each reaction mixture was incubated for 30 min in darkness at ambient temperature, and the absorbance was measured at 517 nm. Standards of Trolox at different concentrations were used (0, 0.5, 1, 1.5, 2, 2.5 and 3.5 mM). Ultrapure water was used as a blank. The radical scavenging activity (RA) against DPPH radicals was assessed according to the following equation (1):

$$RA = [(Abs_{blank} - Abs_{sample}) / Abs_{blank}] \times 100 \quad (1)$$

where Abs_{blank} was the absorbance of the control, and Abs_{sample} was the absorbance of the fruits extract or standard solution.
All assays were conducted in triplicate. Anti-oxidant capacity was expressed in mmol Trolox per 100 g fruits.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). Differences were estimated with a multiple range test using the least significant difference (LSD) at $P < 0.05$.

Results and discussion

Total phenolics contents (TPC)

Total phenolics determined in methanol and ethanol solvents extracts of black and red currants fruits are shown in Tab. 1. The concentration of phenolics in the extracts, expressed as mg GAE/g FW, was dependent on the solvent and method used for extraction. The results indicated significant differences (($P < 0.05$) between cultivars analyzed for each species (*R. nigrum, R. rubrum*) and between methods (variants).

The average total phenolic contents, in the black currant, ranged from 2.52 mg GAE / g FW (variant 4) to 4.6 mg GAE / g FW (variant 3). The average total phenolic contents of 80 % ethanol extracts is at least 0.96 times higher than that of extract 40 % ethanol, 1.1 times higher than that of 80 % methanol extracts and 1.82 times higher than that of extracts in 40 % ethanol by maceration. Results revealed that 80 % ethanol was more efficient in extracting phenolic compounds for black currants fruits.
In the red currant, the average total phenolic content ranged from 0.88 mg GAE / g FW (variant 4) to 1.86 mg GAE / g FW (variant 1). Total phenolic content of 80 % methanol extracts (variant 1) is at least 1.56 times higher than that of extracts in ethanol 40 %, 2 times higher than that of 80 % ethanol extracts and 2.11 times higher than that of extracts in 40 % ethanol by maceration. For red currants, results revealed that 80 % methanol were better solvents in extracting phenolic compounds. It follows that, depending on the species, both used solvent and extraction method are significant factors affecting total phenolic content ($P < 0.05$). Also other authors have established the influence of different extraction solvents and techniques on the phenolic composition in berry fruits and other species (KÄHKÖNEN et al., 2001; JAHANGIRI et al., 2011; MICHIELS et al., 2012; SPIGNO et al., 2007). Generally, solvents such as methanol, ethanol, acetone, propanol and ethyl acetate, have been commonly used for the extraction of phenolics from fresh products. Ethanol is more acceptable for use in food industry. LAPORNIK et al. (2005) indicated that ethanol and methanol extracts of red and black currants contain double amounts of anthocyanins and polyphenols compared to water extracts. The saving of polyphenols from plant materials is depending on solubility of phenolic compounds in the solvent used for the extraction process. According to CACACE and MAZZA (2003), total phenolics in black currants increased with ethanol concentration up to a maximum at about 60 % and then decreased with further increasing in solvent concentration, irrespective of the solvent to solid ratio. Thus, it could be concluded from the results shown in the Tab. 1, that the efficiency of solvents, concentrations of solvent and

Tab. 1: Total phenolics* content of fruits in various cultivars of black and red currant

Genotype		Total phenolic (mg GAE /g FW) in different extracts**			
		Variant 1***	Variant 2	Variant 3	Variant 4
Black currant (*Ribes nigrum*)	'Black down'	$5.70\pm0.27_{d}^{d}$	$3.71\pm0.17_{b}^{b}$	$4.47\pm0.49_{bcd}^{c}$	$2.69\pm0.33_{b}^{a}$
	'Bogatar'	$2.62\pm0.18_{a}^{b}$	$2.38\pm0.08_{a}^{b}$	$2.44\pm0.25_{a}^{b}$	$1.83\pm0.21_{a}^{a}$
	'Tenah'	$4.67\pm0.31_{c}^{b}$	$4.80\pm0.26_{c}^{b}$	$4.93\pm0.52_{d}^{b}$	$2.84\pm0.25_{b}^{a}$
	'Record'	$4.14\pm0.38_{b}^{ab}$	$4.48\pm0.44_{c}^{b}$	$4.69\pm0.44_{cd}^{b}$	$3.53\pm0.36_{c}^{a}$
	'Tinker'	$4.63\pm0.33_{bc}^{b}$	$5.72\pm0.38_{d}^{c}$	$5.89\pm0.56_{e}^{c}$	$2.81\pm0.16_{b}^{a}$
	'Deea'	$3.05\pm0.21_{a}^{a}$	$4.30\pm0.31_{c}^{b}$	$4.04\pm0.42_{bc}^{b}$	$2.67\pm0.18_{b}^{a}$
	'Abanos'	$4.49\pm0.41_{bc}^{b}$	$6.81\pm0.48_{e}^{c}$	$6.48\pm0.56_{e}^{c}$	$1.91\pm0.09_{a}^{a}$
	'Ronix"	$4.12\pm0.25_{b}^{c}$	$3.21\pm0.15_{b}^{b}$	$3.88\pm0.32_{b}^{c}$	$1.92\pm0.23_{a}^{a}$
	Mean	4.17±0.96	4.42±1.36	4.60±1.24	2.52±0.60
Red currant (*Ribes rubrum*)	'Houghton Castle'	$1.92\pm0.15_{b}^{b}$	$1.82\pm0.12_{b}^{b}$	$0.81\pm0.14_{a}^{a}$	$0.93\pm0.09_{a}^{a}$
	'Abundent'	$2.13\pm0.20_{b}^{b}$	$0.96\pm0.16_{a}^{a}$	$1.04\pm0.11_{a}^{a}$	$0.88\pm0.10_{a}^{a}$
	'Rosu timpuriu'	$1.55\pm0.17_{a}^{b}$	$0.81\pm0.07_{a}^{a}$	$0.95\pm0.09_{a}^{a}$	$0.85\pm0.07_{a}^{a}$
	Mean	1.86±0.29	1.19±0.48	0.93±0.14	0.88±0.08

*Average value±standard deviation. **Different subscript letters within the same column indicate significant differences ($P < 0.05$) among cultivars. Different superscript letters within the same row indicate significant differences ($P < 0.05$) among extraction methods. *** Variant 1 (80 % methanol); Variant 2 (40 % ethanol); Variant 3 (80 % ethanol); Variant 4 (40 % ethanol by maceration).

methods are strongly dependent on plant used. The results of this study show that the largest amount of total phenol content from *R. nigrum* was obtained using 80 % ethanol as a solvent while from *R. rubrum* it was obtained using 80 % methanol as a solvent. Phenolics content was different and dependent on cultivars. For black currants, total phenolics varied between 1.83 mg GAE g / FW in cultivar 'Bogatar' and 6.81 mg GAE / g FW in cultivar 'Abanos'. In red currants, total phenolics content variation was between 0.81 mg GAE / g FW in 'Rosu timpuriu' cultivar and 2.13 mg GAE / g FW in 'Abundent' cultivar (Tab. 1). Statistically significant differences ($p \leq 0.05$) were found between of the analyzed cultivars. These differences may be due to genetic factors, and cultivar dependent phenolics contents in currant have been observed by other authors (Djordjević et al., 2010; Pantelidis et al., 2007). It can be concluded that the content of total phenolics is a differentiating factor for the currant cultivars analyzed.

Anti-oxidant capacity

Methanolic and ethanolic extracts derived from currant fruits were evaluated for their anti-oxidant capacity by the DPPH radical scavenging method. Trolox was used as reference standard. Anti-oxidant capacity was expressed in mmol Trolox/100g FW (Tab. 2). Significant differences were found among the free radical scavenging activities of different methods used. Statistically, the interaction between plant materials and solvents significantly ($P < 0.05$) affected the anti-oxidant capacity. Significant differences were found among the free radical scavenging activities of different methods used. Average values of anti-oxidant capacity in black currants ranged from 1.16 mmol Trolox / 100 g FW (variant 4) to 2.35 mmol Trolox / 100 g FW (variant 1). In red currants the variation range was from 0.39 mmol Trolox / 100 g FW (variant 1) up to 2.16 mmol Trolox / 100 g FW (variant 4). It could be concluded from the above results that the antioxidant activities of currant fruit extracts expressed as antiradical power are statistically significant ($P < 0.05$) and they are affected by solvent and methods used for extraction. The highest antioxidant activity was noticed for black currant extracts by 80 % methanol in ultrasonic bath for 55 minutes, centrifuged for 10 minutes, whereas red currant extracted by 40 % ethanol maceration at room temperature for four weeks.

A significant difference ($P < 0.05$) was found among cultivars (Tab. 2) in terms of anti-oxidant capacity; for black currant from 0.82 mmol Trolox / 100 g FW in 'Bogatar' to 2.86 mmol Trolox / 100 g FW in 'Abanos'; for red currant from 0.33 mmol Trolox / 100 g FW to 2.33 mmol Trolox / 100 g FW in 'Abundent'. Cultivars of *R. nigrum* showed higher values for anti-oxidant capacity, compared to cultivars of *R. rubrum*. This is due to anthocyanins content that is higher in the *R. nigrum* species. Borges et al. (2010) showed that black currants contained the highest levels of anthocyanins, and these were responsible for 73 % of the total antioxidant capacity, whereas vitamin C contributed by 18 %. Anti-oxidant capacity of black and red currant polyphenols has already been described. Black currants had the highest antioxidant capacity in the FRAP assay followed by blueberries, raspberries, and red currants, while the lowest value was in cranberries (Borges et al., 2010). Pantelidis et al. (2007) reported the lowest FRAP values (40.7 - 65.1 µmol AsA / g DW) for red currant and gooseberry cultivars.

Anti-oxidant activities were correlated with total phenolic content (Tab. 3). There is an excellent linear relationship (r = 0.84 - 0.98) between values for total phenolic content and anti-oxidant activity. This linear correlation suggested that phenolic compounds in currant largely accounted for its anti-oxidant capacity. Good correlation (r = 0.97 - 0.98) was observed between anti-oxidant activity and total polyphenol content for variant 2 and variant 3, indicating that ethanol, in different concentrations, is a good solvent in obtaining an extract with high anti-oxidant activity of currants (black and red). Similar results have been reported by other researchers. Wang and Lin (2000) found a linear correlation between total anti-oxidant capacity and phenolic content in blackberries (r = 0.961) and raspberries (r = 0.911). A highly significant correlation (0.97) was found between Trolox equivalent antioxidant capacity (TEAC) and total phenolic content in blueberries by Huang et al. (2012).

Tab. 2: Anti-oxidant capacity* of fruits from various cultivars of black and red currant

Genotype		Anti-oxidant capacity (mmol Trolox / 100 g)**			
		Variant 1***	Variant 2	Variant 3	Variant 4
Black currant (*Ribes nigrum*)	'Black down'	$2.54\pm0.14_{c}^{c}$	$1.12\pm0.09_{ab}^{a}$	$2.00\pm0.21_{c}^{b}$	$1.16\pm0.13_{bc}^{a}$
	'Bogatar'	$2.15\pm0.15_{ab}^{c}$	$0.82\pm0.06_{a}^{a}$	$1.16\pm0.16_{a}^{b}$	$1.04\pm0.18_{ab}^{ab}$
	'Tenah'	$2.45\pm0.26_{c}^{c}$	$2.14\pm0.15_{c}^{c}$	$1.80\pm0.13_{bc}^{b}$	$1.12\pm0.10_{b}^{a}$
	'Record'	$2.33\pm0.18_{bc}^{c}$	$2.02\pm0.14_{c}^{b}$	$1.91\pm0.14_{c}^{b}$	$1.39\pm0.14_{d}^{a}$
	'Tinker'	$2.51\pm0.13_{c}^{c}$	$2.66\pm0.28_{d}^{c}$	$2.05\pm0.19_{cd}^{b}$	$1.11\pm0.17_{b}^{a}$
	'Deea'	$2.03\pm0.09_{a}^{c}$	$2.06\pm0.19_{c}^{c}$	$1.53\pm0.14_{b}^{b}$	$1.25\pm0.14_{bcd}^{a}$
	'Abanos'	$2.45\pm0.16_{c}^{b}$	$2.86\pm0.22_{d}^{c}$	$2.32\pm0.23_{d}^{b}$	$1.35\pm0.06_{cd}^{a}$
	'Ronix"	$2.38\pm0.14_{bc}^{d}$	$1.41\pm0.17_{b}^{b}$	$1.88\pm0.13_{c}^{c}$	$0.87\pm0.09_{a}^{a}$
	Mean	2.35±0.22	1.88±0.70	1.83±0.36	1.16±0.19
Red currant (*Ribes rubrum*)	'Houghton Castle'	$0.44\pm0.07_{b}^{a}$	$0.82\pm0.07_{b}^{b}$	$0.72\pm0.08_{b}^{b}$	$2.17\pm0.22_{a}^{c}$
	'Abundent'	$0.33\pm0.04_{a}^{a}$	$0.42\pm0.09_{a}^{a}$	$0.83\pm0.06_{b}^{b}$	$2.33\pm0.21_{a}^{c}$
	'Rosu timpuriu'	$0.40\pm0.03_{ab}^{a}$	$0.49\pm0.04_{a}^{ab}$	$0.59\pm0.04_{a}^{b}$	$1.97\pm0.16_{a}^{c}$
	Mean	0.39±0.06	0.58±0.19	0.71±0.12	2.16±0.23

*Average value±standard deviation. **Different subscript letters within the same column indicate significant differences ($P < 0.05$) among cultivars. Different superscript letters within the same row indicate significant differences ($P < 0.05$) among extraction methods. *** Variant 1 (80 % methanol); Variant 2 (40 % ethanol); Variant 3 (80 % ethanol); Variant 4 (40 % ethanol by maceration).

Tab. 3: Correlation coefficients (r) and coefficient of determination (R^2) of total phenolics and anti-oxidant capacity

Variant	(R^2)	(r)
Variant 1 (80 % methanol)	0.71	0.84
Variant 2 (40 % ethanol)	0.96	0.98
Variant 3 (80 % ethanol)	0.95	0.97
Variant 4 (40 % ethanol by maceration)	0.77	0.87

Conclusion

Analysis of the total phenolic compounds and antioxidant activity of currant fruit extracts showed differences depending on solvent used and extraction method. As it was found, the extracts with higher antioxidant capacity also had higher content of phenolic compounds. It can be established that extracts obtained using ethanol solvents were more effective in radical scavenging activities, compared to extracts obtained using methanol solvents. This is convenient, because when using it in food industry, ethanol is a more suitable solvent. The results obtained are indicating that the analyzed currant species are rich sources of biologically active substances and possess real anti-oxidant activities, for use in food industry, cosmetics, and pharmaceutical industries. Further research would be required for investigations on *in vivo* anti-oxidant activities.

Acknowledgement
This work was partially supported by the grant number 11C /2014, awarded in the internal grant competition of the University of Craiova, Romania.

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